D12 - Translation

MACHINE TRANSLATION OF JP-A-08-034746

CLAIMS

[Claim(s)]
[Claim 1] The drug carrier which consists of micell-like sphingoglycolipid.
[Claim 2] The drug carrier according to claim 1 which is at least one sort chosen from the group which sphingoglycolipid becomes from ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside.
[Claim 3] The manufacturing method of the drug carrier characterized by adding sphingoglycolipid to water or an aquosity medium, and making a micell form.
[Claim 4] The manufacturing method according to claim 3 characterized by adding the organic solvent solution of sphingoglycolipid to water or an aquosity medium, and making a micell form.
[Claim 5] The manufacturing method according to claim 3 or 4 which is at least one sort chosen from the group which sphingoglycolipid becomes from ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside.
[Claim 6] The remedy constituent with which it comes to enclose a drug with the drug carrier which consists of micell-like sphingoglycolipid.
[Claim 7] The remedy constituent according to claim 6 whose drug is the lipophilicity matter or amphiphile.
[Claim 8] The manufacturing method according to claim 6 or 7 which is at least one sort chosen from the group which sphingoglycolipid becomes from ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside.
[Claim 9] The manufacturing method of the remedy constituent characterized by adding sphingoglycolipid and a drug to water or an aquosity medium, and making a micell form.
[Claim 10] The manufacturing method according to claim 9 characterized by adding the organic solvent solution of a drug and making a micell form after adding the organic solvent solution of sphingoglycolipid to water or an aquosity medium.
[Claim 11] The manufacturing method according to claim 9 or 10 whose drug is the lipophilicity matter or amphiphile.
[Claim 12] A manufacturing method given in any 1 term of claims 9-11 which are at least one sort chosen from the group which sphingoglycolipid becomes from ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to those manufacturing methods at the remedy constituent using the drug carrier and this drug carrier which consist of a sphingoglycolipid micell, and a list.
[0002]
[Description of the Prior Art] [currently used as support for the liposome which is a double layer micell to send a drug to a specific target tissue -- D. -- A. tic rel, T.D. heath, and C.M. Korei and B.E. Lyman Biochim.Biophys.Acta 457, and 259-302] (1976). When using liposome as a drug carrier, the factor which should be carefully

taken into consideration [P. which is the incorporation effectiveness of the stability, uniform size, and a water-soluble drug, and the effectiveness of targetting to a target tissue R. coulisse, M. J. Hope, M.B. BARII, T.D. Madden, L.D. Meier, and A.S. JANOFU (1987) "Liposomes : From Biophysics to Therapeutics"(M. J. male fatty tuna edit)39-72 page, Marcel Decker, a New York;L. laser man, and P. MACHII (1987) "Liposomes : From Biophysics to Therapeutics"(M. J. male fatty tuna edit)157 -194 A page, Marcel Decker, New York]. In these, targetting effectiveness is the most important.

[0003] Almost all reports of drug delivery attach importance to carrying out targetting of the liposome enclosure drug to organs other than the liver which is rich in a reticuloendothelial system cell, and a spleen. [D. (1976) (1971) by which the report of carrying out targetting of the liver of 23 is made A. tie rel, T.D. heath, and C.M. Korei and B.E. Lyman Biochim.Biophys.Acta 457, 259-302; J.C. Rogers and S. cone FERUDO Biochem.Biophys.Res.Commun.45, 622-629]. [by which almost all liposome is incorporated by Kupffer cell, and it is processed by these cells, and those limited numbers are incorporated by the parenchymatous cell after reaching liver -- D. -- A. tie rel, T.D. heath, and C.M. Korei and B.E. Lyman Biochim(1976).Biophys.Acta 457, and 259-302]. in order to improve the effectiveness of the orientation of the liposome to a parenchymatous cell -- reduction [Y. of liposome size -- E. Raman -- E. A. Cerny, KR. Patel, E. -- H. Rau and B.J. light (1982) Life Sciences 31, 2061-2071], and those strange method [A. SURORIA and B.K. batch HAWATTO (1977) Biochim.Biophys.Acta497,760-765;S. Yoshioka, Y. BANNO, K. Oakie, T. The processing is complicated although Morita, Y. Ms. paper, and Y. Nozawa (1986) Yakuzaigaku 46 and 247-253] are tried.

[0004]

[Problem(s) to be Solved by the Invention] This invention can be prepared more easily than liposome and aims at offering a drug carrier applicable to a drug delivery system (henceforth "DDS").

[0005]

[Means for Solving the Problem] As a result of repeating research wholeheartedly that the object mentioned above should be attained, sphingoglycolipid formed easily the stability and the small monolayer micell of uniform size, and this invention persons incorporated the lipophilicity matter and amphiphile efficiently by actuation with this easy sphingoglycolipid micell, and found out holding. This shows that matter like the sphingoglycolipid which forms a monolayer micell can use as support in DDS. Moreover, this invention persons found out that a sphingoglycolipid micell was quickly accumulated so much into liver, and ****ed after an intravenous injection there for a long period of time. A sphingoglycolipid micell can use these knowledge as liver orientation support, and building new DDS is shown.

[0006] That is, this invention includes the following invention.

(1) The drug carrier which consists of micell-like sphingoglycolipid.

(2) A drug carrier given in the above (1) which is at least one sort chosen from the group which sphingoglycolipid becomes from ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside.

[0007] (3) The manufacturing method of the drug carrier characterized by adding sphingoglycolipid to water or an aquosity medium, and making a micell form.

(4) A manufacturing method given in the above (3) characterized by adding the organic solvent solution of sphingoglycolipid to water or an aquosity medium, and making a micell form.

[0008] (5) A manufacturing method the above (3) which is at least one sort chosen

from the group which sphingoglycolipid becomes from ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside, or given in (4).
(6) The remedy constituent with which it comes to enclose a drug with the drug carrier which consists of micell-like sphingoglycolipid.
[0009] (7) A remedy constituent given in the above (6) whose drug is the lipophilicity matter or amphiphile.
(8) A manufacturing method the above (6) which is at least one sort chosen from the group which sphingoglycolipid becomes from ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside, or given in (7).
(9) The manufacturing method of the remedy constituent characterized by adding sphingoglycolipid and a drug to water or an aquosity medium, and making a micell form.
[0010] (10) A manufacturing method given in the above (9) characterized by adding the organic solvent solution of a drug and making a micell form after adding the organic solvent solution of sphingoglycolipid to water or an aquosity medium.
(11) A manufacturing method the above (9) whose drug is the lipophilicity matter or amphiphile, or given in (10).
[0011] (12) A manufacturing method given in either of aforementioned (9) - (11) which is at least one sort chosen from the group which sphingoglycolipid becomes from ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside.
if it is a glycolipid containing sphingoid (long-chain amino alcohol of carbon numbers 16-20) as sphingoglycolipid used for this invention -- especially -- a limit -- there is nothing -- for example, ganglioside, RAKUTO ganglioside, globoside, iso globoside, a lactoside, and a neo lactoside -- ganglioside is mentioned preferably. Moreover, as ganglioside, they are GM1, GM2, GM3, GM4, GD1a, GD1b, GT, GT1b, and GT1c, for example. It is mentioned. In this invention, said sphingoglycolipid is used as independent or mixture.
[0012] The drug carrier of this invention can add sphingoglycolipid to water or an aquosity medium, and can prepare it easily by making a micell form. The remedy constituent of this invention can add sphingoglycolipid and a drug to water or an aquosity medium, and can prepare them easily by making a micell form. Under the present circumstances, preferably, it dissolves in organic solvents, such as chloroform, a methanol, ethanol, a pyridine, a tetrahydrofuran, dimethylformamide, the petroleum ether, and the ** ether, other organic solvents which have a polarity comparable as chloroform, or these mixed solutions, and sphingoglycolipid is added to water or an aquosity medium. As said aquosity medium, a physiological saline, a phosphoric-acid buffer physiological salt solution, etc. are mentioned, for example.
[0013] As a drug enclosed with the drug carrier of this invention, if it is the lipophilicity matter or amphiphile, there will be especially no limit, but since the drug carrier of this invention uses liver as a target organ, antitumor agents, such as doxorubicin, etoposide, and fluorouracil, are mentioned preferably. The ratio of the drug carrier in this invention and an enclosure drug can be suitably adjusted by changing the blending ratio of coal of both at the time of forming a micell.
[0014] Sphingoglycolipid is a living body's antibiotic originally, and since it exists also in blood, safety is high. In administration of a large quantity, the activation to the alternative pathway of the complement expected as a side effect is 10 mg/kg. It will be thought that it does not accept if it is administration to extent. therefore, desirable dose as sphingoglycolipid 0.1 - 10 mg/kg it is . The remedy constituent of this invention is usually prescribed for the patient by intravenous administration and

intraarterial administration.
[0015]
[Example] Hereafter, although an example explains this invention concretely, the range of this invention is not limited to the following examples.
[0016] (Example 1)
(1) Cow brain ganglioside was used as an ingredient and (Approach a) ingredient sphingoglycolipid. The ganglioside mixed solution was purchased from Wako Pure Chem Industry. Ganglioside GM 1 was purchased from the sigma chemical company (St. Louis, MO). Egg phosphatidylcholine (PC) came to hand from Avanti! Poral-RIPIZZU (an alabaster, AL). Cholesterol came to hand from NU check PUREPU (ERISHIAN, MN). Sodium lauryl sulfate (SDS) It received from Nakarai Tesuku. Sudan III It received from Wako Pure Chem Industry. The doxorubicin hydrochloride was supplied by Kyowa Hakko Kogyo Co., Ltd. [3H] The cholesteryl hexadecyl ether is E. I. du Pont de Nemours NEN. It received from research products (Boston, MA).
[0017] (b) The inbred strain male C3 H/helium mouse of 6 weeks old of mice came to hand from the Shizuoka laboratory animal breeding farm.
(c) the mol concentration of the measurement ganglioside of ganglioside concentration -- resorcinol-HCl -- law -- [-- K. -- it measured by measuring sialic-acid concentration by Suzuki (1964) LifeSci. and 1227-1233].
[0018] The mol concentration of a ganglioside mixed solution was computed as follows. four sorts of principal components of the ganglioside in cow brain ganglioside pharmaceutical preparation -- GM1 (21%), GD1a (40%), and GD1b (16%) And GT1b (19%) it is -- [-- J. -- C. Samsun (1986) Drugs of Today and 73-107]. It is based on this ratio and is 5mg/ml among a solution. The mol concentration of ganglioside was computed as it is about 2.5 mM, and that sialic-acid concentration was computed as it is 4.8 mM. The observed sialic-acid concentration was well in agreement with calculated value, and was 5.2 mM.
[0019] (d) Sudan III Aliquot of the test sample of each measurement of concentration (0.5ml) After carrying out evaporation to dryness, chloroform 1ml was added. It goes over the solution in 15 minutes at a room temperature, and is bath type sonication equipment (Shibata SU-9TH). Sonication was carried out and centrifugal separation was carried out by 1,900g over 10 minutes at 4 degrees C. Supernatant liquid is collected and it is 0.45 micrometers. Filter (germane sciences, EKIKURO disk 25CR) It filtered. 511nm of filtrate The absorbance which can be set was measured. 10microM in chloroform Sudan III 511nm the absorbance which can be set -- 0.280 it was .
[0020] (e) the measurement doxorubicin concentration of doxorubicin concentration -- BECHA's and others approach [-- N. -- R. BECHA, A.L. Moore, J.G. Bernsten and A. RIU Cancer Chemotherapy Reports (1970) PERT 1 54, and 89-94] were followed substantially, and it measured. When saying simply, the sample solution was diluted with 0.3-N hydrochloric acid in 50% ethanol 20 times, and fluorescence intensity was measured with F-Hitachi 4010 spectrophotofluorometer (excitation wavelength of 470nm; luminescence wavelength of 585nm).
[0021] (f) Gel filtration ganglioside GT1b Physiological saline solution 0.5 ml was applied to sepharose 6B (column size and 1cm x 7.5cm; floor volume, 5.9 ml) which equilibrated with the physiological saline. It was made eluted with a physiological saline and 0.5 ml / fractions were collected. It is the above, and ganglioside concentration was made and measured. SDS a case -- the column of sephadex G-25 -- using it -- SDS You made it eluted with distilled water. SDS It is 236nm about concentration. It measured with the spectrophotometer.

[0022] (g) The preparation ganglioside mixture and Ganglioside GM 1 of a ganglioside micell for drug enclosure were dissolved in chloroform/methanol (1:1, v/v). The ganglioside micell was prepared using ganglioside mixture / ganglioside GM 1 (a mole ratio, 2:1). Evaporation to dryness of the ganglioside mixture is carried out, a physiological saline is added, and it is 50microM about ganglioside concentration. It carried out. At a room temperature, sonication of the solution is carried out, and it carries out centrifugal separation over 1 hour, with bath type sonication equipment, and supernatant liquid is collected, and it is 0.2. mum It filtered with the filter (germane sciences, No.4192).
[0023] (h) The preparation egg PC and cholesterol of liposome were dissolved in chloroform, and liposome was prepared with egg PC / cholesterol (a mole ratio, 2:1). Evaporation to dryness of this mixture was carried out, the physiological saline was added and egg PC concentration was set to 2.5 mM. At a room temperature, sonication of the solution is carried out, and it carries out centrifugal separation over 20 minutes, with horn mold sonication equipment (Branson 250 mold Sonifier, 25% of 2 duty cycle output controls), and supernatant liquid is collected, and it is 0.45 micrometers. It filtered with the filter (germane sciences, No.4184). PC concentration was measured with the analysis kit of phospholipid B-test Wako made from Wako Pure Chem Industry.
[0024] (i) The Sudan III to a ganglioside micell and liposome And the Sudan III of the various amounts dissolved in the enclosure chloroform of the doxorubicin Or the doxorubicin of the various amounts dissolved in the methanol was added in the chloroform / methanol (1:1, v/v) solution of ganglioside, or the chloroform solution of egg PC / cholesterol. After building a ganglioside micell or liposome, as it is the above [these solutions], it filters separately, and they are ganglioside, Egg PC, and Sudan III. And the concentration of the doxorubicin was measured. Before measuring concentration in containing the doxorubicin, the isolation doxorubicin was removed through the solution in G-sephadex 50 column.
[0025] (j) the Sudan III enclosed into a ganglioside micell and liposome and the maintenance freezing-thawing experiment of the doxorubicin -- setting -- the Sudan III where a 10 time mol is superfluous It added in the chloroform / methanol (1:1, v/v) solution of ganglioside mixture, and the chloroform solution of egg PC / cholesterol, and a ganglioside micell and liposome were built. The one half of each sample was frozen at -80 degrees C for 1 hour, and then it maintained for 30 minutes at the room temperature. A thawing solution is filtered and they are ganglioside, Egg PC, and Sudan III. Concentration was measured. In the case of the doxorubicin, the doxorubicin with a superfluous mol is added 5 times in the chloroform / methanol (1:1, v/v) solution of ganglioside mixture, and it is 2.5 to Egg PC. The doxorubicin with a superfluous twice mol was added in the chloroform solution of egg PC / cholesterol. After freezing and thawing, the emitted doxorubicin was separated, and it is the above, and made and measured.
[0026] It sets to a dilution experiment and is Sudan III. The one half of each sample of enclosure ganglioside mixture and liposome was diluted with the physiological saline 5 times, and it maintained at the room temperature for 1 hour. Diluted solution is filtered and they are ganglioside, Egg PC, and Sudan III. Concentration was measured. In the case of the doxorubicin, the doxorubicin with a superfluous mol is added into ganglioside mixture 5 times, and it is 2.5 to Egg PC. The doxorubicin with a superfluous twice mol was added with egg PC / cholesterol. After dilution, before measuring the concentration of the bleedoff doxorubicin, the ultrafiltration by cent RISATO (Centrisart) (the molecular weight cut-off 20,000, SARUTORIASU)

separated the bleedoff doxorubicin from the enclosure object.
[0027] In order to know the effectiveness of a Homo sapiens blood serum over a break through of the doxorubicin, it is the above, and a doxorubicin enclosure ganglioside micell and liposome were made and built. They were incubated at 5 degrees C or 37 degrees C for 16 hours under existence of a Homo sapiens blood serum (the last serum concentration, 25%). After the incubation, the bleedoff doxorubicin was separated, and it is the above, and made and measured.
[0028] (k) It changed slightly, and it is the above substantially, and the doxorubicin enclosure ganglioside micell by ganglioside GM 1, the organ distribution doxorubicin enclosure ganglioside micell of liposome, and liposome were made and built. [3H] cholesteryl hexadecyl ether of the doxorubicin with a superfluous 5 time mol and the amount of traces was added in the chloroform / methanol (1:1, v/v) solution of ganglioside mixture. It is [as opposed to / the case of liposome / Egg PC] 0.1 to the doxorubicin of an equimolecular amount, and Egg PC. [3H] cholesteryl hexadecyl ether of the ganglioside GM 1 of molar quantity and the amount of traces was added in the chloroform solution of egg PC / cholesterol. Although they were filtered after building a ganglioside micell or liposome, it did not let it pass in G-sephadex 50 column. They were administered intravenously to the mouse (0.2ml / mouse), blood was collected and four sorts of organs (liver, a spleen, lungs, and brain) were excised after the perfusion by about 10ml physiological saline by the shown time amount.
[0029] Blood sample (0.2ml) EDTA 3 sodium-salt water-solution 10microl of 100 mM And it incubated at 50 degrees C for 1 hour with Solvable (Solvable) (E. I. du Pont de Nemours NEN research products) 1.0ml, and then EDTA 3 sodium-salt water-solution 0.1 ml of 100 mM and 30% hydrogen-peroxide-solution 0.3 ml were added. It incubated at 50 degrees C for further 1 hour, they were cooled to the room temperature, and then Atomlight (Atomlight) 10(E. I. du Pont de Nemours NEN research products) ml was added. Sonication of the sample is carried out with bath type sonication equipment, blood is solubilized thoroughly, and it is activity Aloka (Aloka) LSC-3500 It measured with the scintillation counter.
[0030] In the case of the organ, they (50mg) were incubated with Solvable (Solvable) 0.5 ml over 3 hours or more at 50 degrees C, and then 0.15ml of hydrogen peroxide solution was added 30%. them -- a room temperature -- 1 hour -- maintaining -- a degree -- Atomlight (Atomlight) -- 10ml was added. Sonication of the sample was carried out with bath type sonication equipment, and the organ was solubilized thoroughly, and activity was measured.
[0031] (2) Ganglioside GT1b investigated by result (a) gel filtration Gel filtration investigated the stability of the ganglioside micell in the concentration of formation versatility of the micell to depend. Ganglioside GT1b It dissolved in the physiological saline without ultrasonicating. ganglioside GT1b from -- the becoming micell showed the single peak by all the examined concentration, and it was shown that the elution profile is the same and a micell is stable irrespective of ganglioside concentration (drawing 1 a). the place which calculated appearance molecular weight -- about 250 kD(s) it was . SDS Although forming a micell was known, appearance molecule sizes differed by examined different concentration (drawing 1 b).
[0032] (b) this invention persons investigated the stability of commercial ganglioside mixture by gel filtration from formation, next the economical viewpoint of the micell by the ganglioside mixture investigated by gel filtration. It dissolved in the physiological saline without ultrasonicating the mixture. The appearance molecule sizes of ganglioside differ by the examined various concentration, and it was shown that commercial ganglioside mixture cannot use it as a drug carrier because of the

instability (drawing 2).
[0033] [T. (1989) by which it is known that insertion of the pure ganglioside GM 1 to liposome will decrease the liposome incorporation by the reticuloendothelial system They are M. allene and C. Hansen and J. root ledge Biochim.Biophys.Acta 981, and 27-35]. So, the ganglioside mixture of marketing which mixed gel filtration with ganglioside GM 1 was followed. 100microM Commercial ganglioside mixture and 50microM The mixture (a mole ratio, 2:1) of ganglioside GM 1 produced the stable micell (drawing 3). The cholesterol and Lynn which are impurity were below limit of detection.
[0034] So, it can be said from an economical viewpoint and the stability as a drug carrier that commercial ganglioside mixture and the mixture (a mole ratio, 2:1) of ganglioside GM 1 are the best combination in inside [having examined until now].
[0035] (c) The effectiveness of enclosure of the drug to the enclosure ganglioside micell of the drug to the ganglioside micell in comparison with liposome was investigated. Sudan III It chose for [the] high hydrophobicity, and the enclosure to a ganglioside micell or liposome was investigated. It is 0.45 micrometers as indicated by the term of "ingredient and (1) Approach". In order to decrease the grain size of liposome so that fully for passing a filter, as compared with the ganglioside micell, the still bigger sonication output was the need per liposome. Ganglioside 100 It is the Sudan III of about 13 molecules per molecule. It encloses with a ganglioside micell at max, and is PC100. It is the Sudan III of about 6 molecules per molecule. It enclosed with liposome at max. This is the Sudan III to a ganglioside micell. The greatest enclosed effectiveness is the Sudan III to liposome. It is shown that it is twice [about] the greatest enclosed effectiveness (drawing 4).
[0036] The doxorubicin which is an amphiphilic antitumor agent was chosen as a common drug. Ganglioside 100 The doxorubicin of about 20 molecules is enclosed with a ganglioside micell per molecule at max, and it is PC100. The doxorubicin of about 4 molecules was enclosed with max per molecule, and it was shown that the greatest effectiveness of enclosure of the doxorubicin to a ganglioside micell is about 5 times the greatest effectiveness of enclosure of the doxorubicin to liposome (drawing 5).
[0037] (d) The stability of maintenance of the maintenance enclosure drug of the enclosure drug in a ganglioside micell and liposome was investigated, and the effectiveness of freezing and thawing to drug maintenance was investigated (table 1). Sudan III When it was used, liposome held the drug more effectively than a ganglioside micell, and, on the other hand, the ganglioside micell held the doxorubicin effectively rather than liposome.
[0038]
[A table 1]
The Sudan III from a ganglioside micell and liposome And the effectiveness of freezing and thawing to a break through of the doxorubicin Sudan III Doxorubicin Support Leakage (%) Leakage (%) Ganglioside micell 13 6 Liposome 0 The effectiveness of the dilution to 18 drug maintenance was investigated (table 2). Sudan III When it was used, liposome held the drug more effectively than a ganglioside micell, and, on the other hand, the ganglioside micell held the doxorubicin effectively rather than liposome.
[0039]
[A table 2]
The Sudan III from a ganglioside micell and liposome And the effectiveness of the dilution to a break through of the doxorubicin Sudan III Doxorubicin Support

Leakage (%) Leakage (%) Ganglioside micell 32 16 Liposome 20 As a result of examining the effectiveness of a Homo sapiens blood serum over 21 drug maintenance, it was shown that a ganglioside micell and liposome hold 90% or more of the drug first enclosed after the incubation of 16 hours at 37 degrees C under 25% of existence of a Homo sapiens blood serum (table 3).

[0040]

[A table 3]

ガングリオシドミセル及びリポソームからのドキソルビシンの漏出に対するヒト血清の効果

温　度 (℃)	ドキソルビシンの漏出率（%）	
	ガングリオシドミセル	リポソーム
5	9	9
37	9	8

[0041] (e) the doxorubicin enclosure ganglioside micell by ganglioside GM 1, the ganglioside micell in the organ distribution organ of liposome, and distribution of liposome -- as a marker -- [3H] cholesteryl hexadecyl ether [-- J. -- it investigated using T.P. Di Luc Seng, H.W.M. mho SERUTO and G.L. SHIERUHOFU Biochim.Biophys.Acta 931, and 33-40] (1987).

[0042] In consideration of total recovery being called the ratio of the sum total of activity collected from the blood and four sorts of organs to the first activity, the total recovery (81 - 99%) from a ganglioside micell was higher than the total recovery (42 - 58%) from liposome (drawing 6 a). In the case of the ganglioside micell, about 60% of the first activity was collected in liver in 30 minutes after an intravenous injection, and 79% was collected in 24 hours. However, in the case of liposome, about 7% was collected in liver slightly [the first activity] in 30 minutes after the intravenous injection, and 48% was collected in 24 hours (drawing 6 a). In are recording of the activity in other organs, a spleen, lungs, and a brain, there was no difference remarkable between a ganglioside micell and liposome (drawing 6 b).

[0043] (3) [by which the attempt which is going to use a micell as an examination drug carrier was restricted to the activity of the liposome which forms a double layer micell (lamellae) -- D. -- A. tie rel, T.D. heath, and C.M. Korei and B.E. Lyman Biochim.Biophys.Acta 457, and 259-302] (1976). although it is known that the monolayer micell by surfactant like SDS will incorporate coloring matter -- [-- K. -- [which the report of the activity of Suzuki (1964) Life Sci., 1227-1233], and the monolayer micell as a drug carrier did not have -- D. -- A. tie rel, T.D. heath, and C.M. Korei and B.E. Lyman Biochim.Biophys.Acta 457, and 259-302] (1976). It is shown for the first time that the monolayer micell of sphingoglycolipid, such as ganglioside, can use this invention as a stable liver orientation drug carrier of small size.

[0044] It is thought that a monolayer micell is not suitable as a drug carrier for the instability of the low maintenance capacity and a micell. However, the appearance size of a monolayer micell is smaller than the appearance size of liposome, And the critical micelle concentration (cmc) The aggregate formed automatically in a top is the thing of the size of homogeneity. a certain thing -- [K. -- SHINODA [(1963)] -- "Colloidal Surfactants: Some Physicochemical Properties" (K. SHINODA) T. NAKAGAWA, B. TAMAMUSHI, and 1 - 96 pages of T. ISEMURA edits, It has an advantage to liposome in respect of Academic Press and New York]. Liposome is SDS. Although it is known that it is more stable than the usual monolayer micell [like

], the size heterogeneity is disadvantageous.
[0045] cmc of ganglioside irrespective of molecular species -- about 70-100 (1972) muM it is -- [to which things are reported -- H. -- C. YOHE and A. low ZEMBERUGU Chem.Phys.Lipids.9, and 279-294]. For drawing 1 a, the appearance molecule size of a ganglioside micell is cmc of ganglioside. It is shown by up-and-down [both] that it was the same. The globular form hydrophobic gestalt surrounded by the sugar network structure accompanied by one or more sialic acids which ganglioside has combined with each ceramide chain which branched is small, and this shows that a uniform micell is formed. SDS cmc [[K. (1963) reported to be about 8 mM(s) SHINODA"Colloidal Surfactants : Some Physicochemical Properties"1(K. SHINODA, T. NAKAGAWA, B. TAMAMUSHI, and T. ISEMURA edit)-96 page, Academic Press, and New York]. Drawing 1 b shows existence of a single peak by 100 mM, and has the additional peak which is equivalent to molecular weight lower than the first thing by both 6mM and 4mM(s), and, for this, appearance molecule size is SDS. Changing according to concentration is shown. These results to a ganglioside micell is SDS. It can bc said that it is more stable than a micell and can use as a drug carrier.
[0046] Based on the result shown in drawing 1 a, ganglioside micells gather and this invention persons are about 120. What forms the globular form configuration which consists of a ganglioside molecule was presumed. if it becomes what -- the appearance molecular weight -- about 250 kD(s) it is -- it is because the hydrophobic part is the diameter of about 4nm. the multilamellar liposome often used as a drug carrier -- usual distributed approach [-- F. -- Olson, C.A. FUNTO, F.C. AZOKA, a W.J. bail and D. papa HAJO porous Biochim.Biophys.Acta 557, and 9-23] -- diameter (1979) 0.1 mum -3micrometer It is obtained as an uneven vesicle of the range. [reported for large liposome to be easily caught by the reticuloendothelial system -- Y. -- E. Raman, E.A. Cerny, KR. Patel, E.H. Rau and B.J. light (1982) Life Sciences 31, and 2061-2071 --] . In this way, it can be said that the ganglioside micell which has uniform size is superior to liposome so that it may be explained later.
[0047] The stability of commercial ganglioside mixture was investigated from an economical viewpoint. The micell formed with the mixture is single ganglioside kind GT1b, although size was small. It was not more stable than the formed micell (drawing 2). In order to know whether the instability of the micell formed with commercial ganglioside mixture originates in mixing of two or more ganglioside kinds, gel filtration was performed using the mixture of four sorts of pure gangliosides (GD1a, GD1b, GT1b, GM1) which are the principal components of commercial ganglioside mixture. Consequently, the single peak and the same elution profile were shown irrespective of concentration (drawing 7). So, what a difference of the stability of commercial ganglioside mixture and the mixture of four sorts of pure gangliosides will be because the existence of contamination in the former is suggested. This instability in the micell of commercial ganglioside mixture can be easily solved by addition of the ganglioside GM 1 of the mole ratio of 2 to 1 (drawing 3).
[0048] It is necessary to observe the data that a ganglioside micell is about 5 times as effective as liposome about enclosure of the doxorubicin (this is bulky a little because of the existence of the sugar in the molecule, and a hydrophobic part, and size is abbreviation 0.7nm x 1.2nm) which is an amphiphilic antitumor agent (drawing 5). For this data, liposome is Sudan III. It is shown that hold the lipophilicity matter [like] preferentially and a ganglioside micell holds amphiphile like the doxorubicin preferentially on the other hand (tables 1 and 2). A ganglioside micell can enclose into

a molecule the drug which has a bulky hydrophobic part of a certain kind. A ganglioside micell is Sudan III. The reason for enclosing the doxorubicin effectively can be explained as follows. The anthraquinone part of the doxorubicin meets with the ceramide part of a ganglioside molecule, and the hexose part which is a hydrophilic part of the doxorubicin combines with a part for the sugar part of the molecule by hydrogen bond simultaneously.

[0049] The ganglioside micell containing an enclosure drug was resistance to dilution at freezing and thawing, and a list (tables 1 and 2). Moreover, it holds the doxorubicin under existence of a Homo sapiens blood serum effectively like liposome (table 3). In these results, a ganglioside micell shows that after enclosure of a drug holds the physical stability. About targetting, a ganglioside micell is promptly accumulated into liver after the intravenous administration, and the accumulated dose increases it slightly after that (drawing 6 a). The total recovery (81 - 99%) of the activity from a ganglioside micell was higher than the total recovery (42 - 58%) from liposome (drawing 6 a). These results show that it is useful as a drug carrier to which a ganglioside micell uses liver as a target organ. A liver cell consists of a parenchymatous cell and a non-parenchymatous cell. Knowledge [Y. that small liposome is easily caught by the parenchymatous cell E. Raman, E. A. Cerny, KR. Patel, E.H. Rau, and B.J. light (1982) Life Sciences 31, 2061-2071], and the size of the aperture between liver endothelial cells -- about 100nm WISSE it is -- [E. -- R. Di ZANGA and R. JAKONZU (1982) "Sinusoidal Liver Cells" (D. L. NOKU and E. WISSE edit) 61-67 Page, ERUSEBIA biotechnology medical treatment press, Based on the result that Amsterdam] and a drug need to pass through these gaps, it can be said that it may reach a parenchymatous cell easily since a ganglioside micell is smaller than any of liposome and an aperture. [to which the receptor of a galactose is reported about the parenchymatous cell -- A. -- G. Morel, R.A. IRUBIN, I. Der Stern reeve, and I.H. SHAIMBERUGU (1968) -- J.Biol.Chem.243, a 155-159;H.H. span jar and G.L. SHIERUHOFU Biochim.Biophys.Acta 734, and 40-47] (1983). Ganglioside GM1 molecule and GD1b in a ganglioside micell [A. (1977) to which the galactose residue in the nonreducible end of both molecules can participate in prehension of the micell by the parenchymatous cell again They are SURORIA and B.K. batch HAWATTO Biochim.Biophys.Acta497,760-765].

[0050] It is necessary to pay attention to two problems for the application on clinical [of a ganglioside micell]. [H. (1993) whose ganglioside of the first problem is activating the alternative pathway of human complement cascade They are OOSHIMA, G. SOMA and D. Mizuno Int.Immunol.5, and 1349-1351]. [which is that Guillain Barre syndrome accompanied by a motor nerve failure produces the second problem after ganglioside intake -- N. -- YUUKI, T. Taki, F. INAGAKI, T. KASAMA, M. Takahashi, K. SAITOU, S. pewter and T. MIYATAKE J.Exp.Med.178, and 1771-1775 --] . (1993). The former problem can be solved by adjusting ganglioside concentration. Human alternative pathway is 50microM. [H. (1993) which is not activated by ganglioside by the concentration of the following OOSHIMA, G. SOMA and D. Mizuno Int.Immunol.5, 1349-1351]. The latter problem is histocompatibility antigen HLA-B35. It can solve by avoiding administration of the ganglioside to the male and woman who have. [which is because significant relation is between that disease and this antigen on statistics -- N. -- YUUKI, S. Sato, T. ITOU and T. MIYATAKE (1991) Neurology 41, and 1561-1563 --] .

[0051] As a conclusion, it was first proved [persons / this invention] as a result of the high effectiveness at the time of the micell of sphingoglycolipid, such as ganglioside,

enclosing and holding a to some extent bulky molecule in the easy thing of the preparation, its uniform size, and a list small that liposome was excelled as a drug carrier. Moreover, it was proved [persons / this invention] that sphingoglycolipid, such as ganglioside, was useful as a liver orientation drug carrier.

[0052] (Example 1 of a trial) It is 2microg as doxorubicin to five acute toxicity test mice (C3 H/helium). Ganglioside GM1 micell to contain 400microg/kg was administered intravenously. Consequently, the side effect was accepted in no mice prescribed for the patient. From this result, it is expected that fifty percent lethal dose of the doxorubicin content remedy constituent concerned is 1mg/kg or more.

[0053]

[Effect of the Invention] According to this invention, rather than liposome, it can prepare easily and a drug carrier applicable to DDS can be offered.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] Ganglioside GT1b And SDS It is drawing showing the result of gel filtration. a. is ganglioside GT1b. The case where it applies to the column of sepharose 6B is shown. b. is SDS. The case where it applies to the column of sephadex G-25 is shown.

[Drawing 2] It is drawing showing the result of the gel filtration of the ganglioside mixture of marketing by sepharose 6B.

[Description of Notations]

O Commercial ganglioside mixture concentration 100microM

- Commercial ganglioside mixture concentration 12.5 muM

[Drawing 3] It is drawing showing the result of the gel filtration of the ganglioside mixture of marketing by sepharose 6B, and the mixture of GM1.

[Description of Notations]

O GM1 100microM

- GM1 100microM+ ganglioside mixture 100microM

** GM1 100microM+ ganglioside mixture 50microM

** GM1 50microM+ ganglioside mixture 100microM

[Drawing 4] The Sudan III to a ganglioside micell and liposome It is drawing showing enclosure. An axis of abscissa is Sudan III. The mole ratio of pair ganglioside or Egg PC is shown. An axis of ordinate is ganglioside. The Sudan III enclosed with per 100 molecules or egg PC 100 molecule The number of molecules is shown.

[Description of Notations]

O Ganglioside micell

- Liposome

[Drawing 5] It is drawing showing enclosure of the doxorubicin to a ganglioside micell and liposome. An axis of abscissa shows the mole ratio of doxorubicin pair ganglioside or Egg PC. An axis of ordinate is ganglioside. The number of the doxorubicin molecules enclosed with per 100 molecules or egg PC 100 molecule is shown.

[Description of Notations]

O Ganglioside micell

- Liposome

[Drawing 6] It is drawing showing the organ distribution of the doxorubicin enclosure ganglioside micell by the ganglioside GM 1 observed by the activity of [3H]

cholesteryl hexadecyl ether as a marker, and liposome. A notation shows the average of three measurement.
[Description of Notations]
O The sum total about a ganglioside micell
** Liver about a ganglioside micell
Blood about a ** ganglioside micell
<> Spleen about a ganglioside micell
The lungs and brain about a ** ganglioside micell
- The sum total about liposome
** Liver about liposome
Blood about ** liposome
<> Spleen about liposome
The lungs and brain about liposome
[Drawing 7] It is drawing showing the result of the gel filtration of the mixture of the pure ganglioside of marketing by sepharose 6B.